Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 4360–4364, July 1985
Biochemistry

Isolation of cDNA for a human granulocyte–macrophage colony-stimulating factor by functional expression in mammalian cells

(helper T cells/growth factor/gene cloning/transient expression/DNA sequence analysis)

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Communicated by George E. Palade, March 22, 1985

ABSTRACT **A cDNA sequence coding for a human granulocyte–macrophage colony-stimulating factor has been isolated from cDNA libraries prepared from mRNA derived from concanavalin A-activated human T-cell clones. The libraries constructed in the pcD vector system were screened by transfecting COS-7 monkey cells with DNA pools to express the products encoded by full-length cDNA inserts. By assaying the cell supernatants, we identified clones encoding a factor that stimulates the formation of granulocyte and macrophage colonies from human progenitor cells. These results demonstrate that identification of full-length cDNAs for many colony-stimulating factors may be achieved entirely on the basis of detecting the functional polypeptide produced in mammalian cells.**

T lymphocytes regulate the growth and differentiation of certain lymphopoietic and hematopoietic cells through the action of soluble secreted protein factors known as lymphokines (1). After activation by either antigen or lectin (2, 3), helper T lymphocytes are capable of producing a variety of lymphokines, including colony-stimulating factors (CSFs). CSFs are a family of glycoproteins involved in the control of proliferation or differentiation of hematopoietic cells (4, 5). CSFs are made not only by mitogen-activated T cells but also by a variety of other cell types, including fibroblasts, macrophages, and endothelial cells (6).

CSFs can be recognized *in vitro* by their ability to stimulate the formation of colonies of differentiated cells in semisolid cultures of bone marrow stem cells. Several such factors from murine sources have been characterized, each capable of stimulating the development of different types of colonies. cDNA clones encoding two such factors have recently been isolated (7–9). One of these factors, interleukin 3 (IL-3) or multi-CSF, has the capacity to stimulate a wide range of colony types (10). The other, granulocyte–macrophage CSF (GM-CSF), appears to be more limited in its action and generates only granulocyte and macrophage colonies (7). Similar factors from several human sources have been described and partially purified, but in general these have been less well characterized. Despite the report that biologically active human GM-CSF could be produced by translation of mRNA in *Xenopus laevis* oocytes (11), further definition of the various human CSF activities remains hampered by the limited availability of polypeptides in biochemically pure form.

Using a pcD cDNA library, we have recently developed a screening procedure employing transfection of plasmid DNAs into mammalian cells, followed by assaying the transfected cell supernatants for biological activities of interest (12). We have adopted this approach to screen a pcD cDNA library prepared with mRNA from a concanavalin A (Con A)-activated human T-cell line for cDNA clones that can express CSF activity in COS-7 monkey cells. In this manner we have identified one class of human cDNA clones encoding a granulocyte–macrophage colony stimulating activity. Nucleotide sequence analysis of these clones reveals substantial homology with mouse clones for GM-CSF (7).

MATERIALS AND METHODS

Cell Lines and Isolation of mRNA. The human T-cell lines T7 (obtained from G. Nabel; Dana-Farber Cancer Institute, Boston, MA) and 5C10 and human peripheral blood lymphocytes (supplied by H. Pohlit and J. Banchereau; Unicet Laboratories, Lyon, France) were stimulated with Con A at 2 μg/ml, 5 μg/ml, and 7 μg/ml, respectively. All cells were harvested 4 hr after addition of Con A. Total cellular RNA was extracted from the cells by using the guanidium thiocyanate method (13), and poly(A)$^+$ RNA was selected by oligo(dT) cellulose chromatography.

Construction of cDNA Libraries. A pcD cDNA library was established with mRNA from T7 cells according to the procedure of Okayama and Berg (14). Using the same vectors, similar cDNA libraries were constructed with 5C10 and peripheral blood lymphocyte mRNA with one modification. The pcDV1 plasmid used in these cases contains an *Nsi* I restriction site at the previous location of the *Kpn* I site (14). Each of the cDNA libraries contained a minimum of 10^5 independent clones.

Screening of cDNA Libraries by Transfection. Approximately 10^4 independent bacterial colonies were picked randomly from the T7 cDNA library and propagated individually in wells of microtiter dishes. Pooled cultures representing 48 clones were grown and plasmid DNA was isolated for the initial phase of screening. COS-7 monkey cells were transfected with plasmid DNA, using DEAE-dextran as described previously (8). After a 4-hr incubation at 37°C, the cells were washed and Dulbecco-modified Eagle's medium (DME) containing 150 μM chloroquine was added as described previously (12). The chloroquine treatment increases the efficiency of the transfection. After 3 hr this was replaced with DME containing 4% fetal calf serum. Seventy-two hours later the medium was collected and assayed in a colony formation assay.

Screening of cDNA Libraries by Hybridization. We isolated functional mouse GM-CSF cDNA clones from pcD libraries constructed with mRNA from Con A-activated helper T cells (to be described elsewhere). A *Pst* I/*Aha* III fragment, isolated from the mouse GM-CSF cDNA, was labeled by nick

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Abbreviations: Con A, concanavalin A; CSF, colony-stimulating factor; GM-CSF, granulocyte–macrophage CSF; IFN-γ, γ interferon; IL-2, interleukin 2; IL-3, interleukin 3.

translation (1×10^8 cpm/μg) and used to probe nitrocellulose filters containing random colonies from 5C10 and peripheral blood cDNA libraries. Low stringency hybridization conditions were used: 6× SSPE buffer (1× SSPE = 180 mM NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) (15), 50% (vol/vol) formamide, 0.1% sodium dodecyl sulfate, carrier tRNA at 100 μg/ml, overnight at room temperature. The filters were washed with 1.5 mM sodium citrate, pH 7.0/15 mM NaCl/0.1% sodium dodecyl sulfate at 42°C.

***In Vitro* Colony-Forming Assays.** Colony-forming assays were performed with either human bone marrow cells obtained from patients with nonhematologic disease or with human cord blood cells (16, 17). Approximately 10^5 nonadherent cells from bone marrow or cord blood were added to 35-mm Petri dishes in a total volume of 1 ml of modified Iscove's medium (10) supplemented with 0.9% methylcellulose containing the test sample at a concentration not greater than 30% of the total volume. Cultures were incubated for 3 days, then 1 unit of human erythropoietin was added. Colonies were counted after 10–14 days. The cellular composition of colonies was determined after applying individual colonies to glass slides and staining with Wright–Giemsa stain.

DNA Sequence Analysis. Nucleotide sequences were determined by using the phage M13 dideoxy chain termination method (18) or a modified procedure of Maxam and Gilbert (19, 20).

RESULTS

Evaluation of CSF Activities Produced by T-Cell Clones. Initial experiments showed that the human helper T-cell clones T7 and 5C10 produce and secrete interleukin 2 (IL-2) and γ interferon (IFN-γ) activities after stimulation by Con A. Using pcD cDNA libraries prepared with mRNA isolated from Con A-stimulated T7 or 5C10 cells, we isolated full-length human IL-2 and IFN-γ cDNA clones (unpublished results). The frequency of IL-2 and IFN-γ cDNA clones was estimated to be approximately 0.1–0.5% of the total clones in the T7 library. Individual clones were identified that produced functional IL-2 or IFN-γ when transfected into COS-7 monkey cells. Furthermore, using semisolid cultures of human bone marrow or cord blood cells, we have also confirmed that supernatants from 5C10 cells stimulate the proliferation of neutrophils, macrophages, eosinophils, and erythroid elements. We therefore reasoned that cDNA libraries for either of these T-cell clones could be screened by transfection to identify cDNA clone(s) encoding factors capable of stimulating colony formation by progenitor cells in human bone marrow or cord blood.

Screening the cDNA Library for CSFs by Transfection in Monkey Cells. To identify the CSF cDNA clones, bacterial clones from the pcD T7 cDNA library were pooled into groups of 48 clones. Plasmid DNA from each pool of clones was transfected into COS-7 cells, using DEAE-dextran. After 72 hr, the supernatants from the transfected cells were assayed for CSF activity, using semisolid cultures of bone marrow or cord blood cells. Approximately 2000 bacterial clones were screened in this manner. Four pools (groups 1, 3, 7, and 14) were found to stimulate the formation of colonies (Table 1).

Each of these groups of clones was divided into subpools, each containing eight of the original clones. From each of the original positive pools, one subpool of eight clones was positive in the transfection assay with the exception of group 7A, which yielded two positive subpools. Each of the plasmid clones contained in groups 3-8, 7-1, 7-4, and 14-1 was then transfected individually into COS-7 cells. One clone from each group of eight was active in producing CSF activity. Thus, it appears that each of these cDNA inserts contains sufficient information to direct the synthesis of a functional CSF polypeptide. Restriction endonuclease analysis showed that all of these clones share essentially the same structure (see Fig. 2*B*).

Table 1. Assay of colony-stimulating activity in plasmid DNA pool transfection supernatants

Screening	Cells screened	Results
First	40 pools of 48 clones (1–20, A and B)	4 positive pools (1A, 3B, 7A, and 14A)
Second	Subpools of 8 clones	5 positive pools (1-5, 3-8, 7-1, 7-4, and 14-1)
Third	Individual clones	Active clones (3-8a, 7-1a, 7-4d, and 14-1e)

In the first screening, plasmid DNAs for 40 pools of 48 random cDNA clones were transfected into COS-7 cells, and the cell supernatants were assayed for colony-stimulating activity. Positive signals were detected with four pools, identified as those cultures containing more than 20 clusters (20–50 cells) per 10^5 cells added to the culture. In the second screening, subpools each containing eight cDNA clones were tested in a similar fashion. Positive signals ranged from 50 clusters to 100 small colonies (50–150 cells) per 10^5 cells. The third screening with individual clones produced results ranging from 60 to 200 colonies (greater than 150 cells) per 10^5 cells.

Identification of Human CSF cDNA Clones by Hybridization with Mouse GM-CSF cDNA Probe. pcD-cDNA libraries prepared from the 5C10 cell line and from Con A-stimulated peripheral blood lymphocytes were screened separately for possible homology with a mouse GM-CSF cDNA clone isolated from a mouse helper T-cell cDNA library and essentially identical to a clone isolated from a mouse lung cell cDNA library (ref. 7; unpublished results). A ^{32}P-labeled cDNA probe derived from the mouse GM-CSF cDNA insert was used to screen filters containing random colonies from 5C10 or peripheral blood lymphocyte-derived cDNA libraries. After the filters had been washed under low-stringency conditions (42°C), clones hybridizing with the mouse GM-CSF cDNA probe were identified (Fig. 1). They represent approximately 0.5% and 0.05% of 5C10 and peripheral blood lymphocyte libraries, respectively. When plasmid DNA for one of the longest hybridizing clones from each cDNA library was transfected into COS-7 cells, the cell supernatant contained CSF activity (Table 2). Analysis with restriction endonucleases showed that each of the hybridizing clones is identical in structure to the active T7-derived cDNA clones identified by transfection. These results suggest that human T cells express a unique class of CSF that shares homology with mouse GM-CSF. We designate these clones as human GM-CSF cDNA clones.

Nucleotide Sequence of the Human GM-CSF cDNA. Fig. 2 shows the structure of pcD-hGM-CSF and the restriction

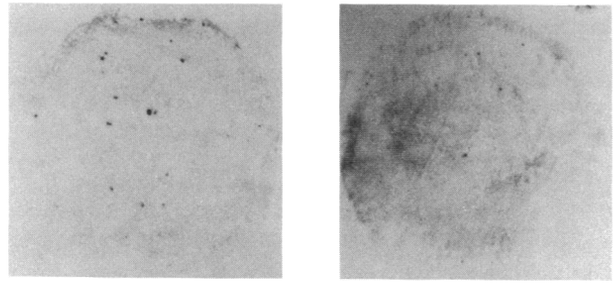

FIG. 1. Screening of human cDNA libraries with a probe from the mouse GM-CSF cDNA. Nitrocellulose filters containing approximately 5000 random clones were probed with a nick-translated *Pst* I/*Aha* III fragment under low-stringency hybridization conditions. These are examples of clones from the 5C10 cDNA library (*Left*) and the peripheral blood library (*Right*).

Table 2. Colony formation by transfected COS-7 cell supernatants

Clone	Source	Colonies per 10^5 cells: Human bone marrow	Human cord blood	Mouse bone marrow
Mock	—	<5	<5	<5
E1-11	E1	ND	0	230
3-8a	T7	ND	123	ND
7-1a	T7	56	186	0
7-4d	T7	ND	168	0
14-1e	T7	65	85	0
17-1	5C10	ND	84	ND
204	PB	41	120	0
CM	5C10	29	99	ND

Cells were transfected and supernatants were collected after 72 hr, then assayed in duplicate 1-ml cultures containing 10^5 human bone marrow, human cord blood, or murine bone marrow cells. The numbers of colonies containing more than 150 cells were determined after 14 days for human cultures and after 7 days for murine cultures and are reported as the mean value for the duplicate cultures. Controls were supernatant from mock-transfected COS-7 cells, supernatant from cells transfected with full-length murine GM-CSF cDNA clone E1-11 (unpublished), or supernatant from Con A-stimulated human T-cell clone 5C10. ND, not done; PB, peripheral blood; CM, conditioned medium.

endonuclease cleavage map of one of the longest cDNA inserts that produces biologically active GM-CSF (clone 3-8a). The DNA sequence of the cDNA insert of clone 3-8a was determined and is shown in Fig. 3. The cDNA insert contains a single open reading frame, with the first ATG located 33 nucleotides from the 5′ end followed by 144 codons before the termination triplet TGA at nucleotide positions 465–467. The deduced amino acid sequence for this human cDNA clone is highly homologous with that for mouse GM-CSF (Fig. 4; ref. 7; unpublished results).

Biological Activity of Human GM-CSF Expressed in Monkey Cells. During the initial screening of the pooled cDNA clones, the colonies stimulated by the transfected COS-7 cell supernatants were not analyzed in detail. When individual functional clones were identified from both the T7 and 5C10 cDNA libraries, supernatants of COS-7 cells transfected with these clones were assayed in cultures of human bone marrow or cord blood cells. The resulting colonies (see Fig. 5 *Upper*) were micropipetted, applied to glass slides, and stained with Wright-Giemsa for morphologic analysis. The results, summarized in Table 3, show that all the clones express in COS-7 cells a product that stimulates the formation of granulocyte

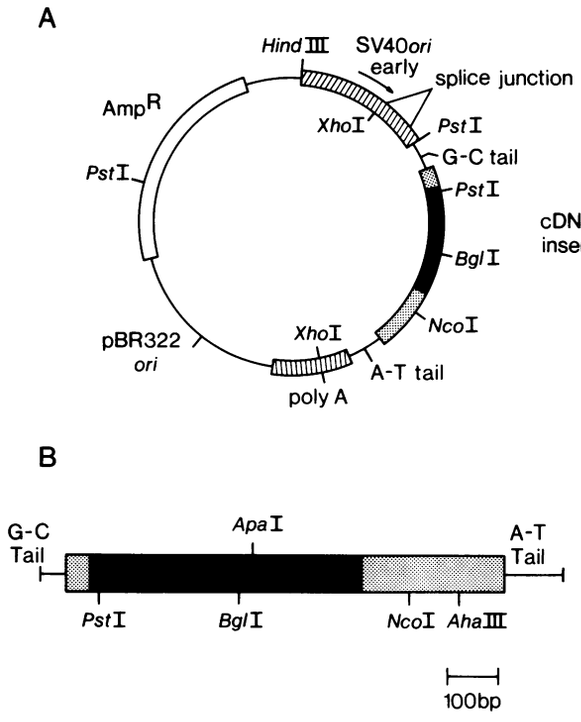

FIG. 2. (*A*) General diagram of pcD-hGM-CSF, the plasmid carrying the functional GM-CSF cDNA insert. The cDNA insert extends from the G·C tail to the A·T tail and contains the indicated restriction endonuclease cleavage sites; the coding region is heavily shaded, and the noncoding regions are lightly shaded. The direction of transcription from the simian virus 40 (SV40) promoter is indicated by the arrow. The structure of the remainder of the plasmid is as described in ref. 14. (*B*) Diagram of the insert in clone 3–8a. bp, Base pairs.

```
        10          20          30                          50                  65
ACACAGAGAG AAAGGCTAAA GTTCTCTGGA GG ATG TGG CTG CAG AGC CTG CTG CTC TTG GGC ACT GTG GCC TGC
                                     MET Trp Leu Gln Ser Leu Leu Leu Gly Thr Val Ala Cys
    80                  95                  110                 125                 140
AGC ATC TCT GCA CCC GCC CGC TCG CCC AGC CCC AGC ACG CAG CCC TGG GAG CAT GTG AAT GCC ATC CAG
Ser Ile Ser Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His Val Asn Ala Ile Gln
            155                 170                 185                 200
GAG GCC CGG CGT CTC CTG AAC CTG AGT AGA GAC ACT GCT GCT GAG ATG AAT GAA ACA GTA GAA GTC ATC
Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile
215             230                 245                 260                 275
TCA GAA ATG TTT GAC CTC CAG GAG CCG ACC TGC CTA CAG ACC CGC CTG GAG CTG TAC AAG CAG GGC CTG
Ser Glu Met Phe Asp Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln Gly Leu
        290                 305                 320                 335                 350
CGG GGC AGC CTC ACC AAG CTC AAG GGC CCC TTG ACC ATG ATG GCC AGC CAC TAC AAG CAG CAC TGC CCT
Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met Met Ala Ser His Thr Lys Gln His Cys Pro
                365                 380                 395                 410
CCA ACC CCG GAA ACT TCC TGT GCA ACC CAG ATT ATC ACC TTT GAA AGT TTC AAA GAG AAC CTG AAG GAC
Pro Thr Pro Glu Thr Ser Cys Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp
    425             440                 455             467             480         490
TTT CTG CTT GTC ATC CCC TTT GAC TGC TGG GAG CCA GTC CAG GAG TGA  GACCGGCCAGATG AGGCTGGCCA
Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu  .
        500         510         520         530         540         550         560         570
AGCCGGGGAG CTGCTCTCTC ATGAAACAAG AGCTAGAAAC TCAGGATGGT CATCTTGGAG GGACCAAGGG GTGGGCCACA
        580         590         600         610         620         630         640         650
GCCATGGTGG GAGTGGCCTG GACCTGCCCT GGGCACACTG ACCCTGATAC AGGCATGGCA GAAGAATGGG AATATTTTAT
        660         670         680         690         700         710         720         730
ACTGACAGAA ATCAGTAATA TTTATATATT TATATTTTTA AAATATTTAT TTATTTATTT ATTTAAGTTC ATATTCCATA
        740         750         760         770         780         789
TTTATTCAAG ATGTTTTACC GTAATAATTA TTATTAAAAA TAGCTTCTAA AAAAAAAAA
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FIG. 3. Nucleotide sequence and predicted amino acid sequence for human GM-CSF. The nucleotide sequence begins with position 1 at the first nucleotide following the oligo(dG) segment. The amino acid sequence begins with the first in-phase ATG codon for the single long open reading frame. The underlined amino acids indicate the locations of potential N-glycosylation sequences (Asn-Xaa-Thr or Asn-Xaa-Ser).

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                                    10                                      20
MET Trp Leu Gln Ser Leu Leu Leu Leu Gly Thr Val Ala Cys Ser Ile Ser Ala Pro Ala Arg Ser Pro
                Asn         Phe         Ile     Val Tyr     Leu             Thr

                        30                                      40
Ser Pro Ser Thr Gln Pro Trp Glu His Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser
Ile Thr Val     Arg         Lys         Glu         Lys         Leu Asn         Asp Asp Met

            50                                  *   60      *   *       *       *   *   *
Arg Asp Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe Asp Leu Gln Glu Pro
Pro Val     Leu Asn     Glu Val     Val     |       Phe                 |
                                        Ser|Asn                      Lys|Lys

70                                      80                                      90
Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly
        Val                 Lys Ile Phe Glu                     Asn Phe

                            100                                     110
Pro Leu Thr Met Met Ala Ser His Thr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser Cys Ala Thr
Ala     Asn     Thr         Tyr     Gln Thr Tyr                             Asp     Glu

                120                                     130
Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp Phe Leu Leu Val Ile Pro Phe Asp Cys
    Val Thr     Tyr Ala Asp     Ile Asp Ser         Thr         Thr Asp             Glu

    140
Trp Glu Pro Val Gln Glu
Lys Lys     Ser     Lys
```

FIG. 4. Comparison of the deduced amino acid sequence for human GM-CSF with that predicted for mouse GM-CSF. Upper line is the complete human sequence. The underlined amino acid residues are identical between mouse and human GM-CSF, while the residues in the mouse sequence that are different are shown below the human sequence. Adjustments were made to maximize the homology between the two sequences; there are two positions (57–58 and 65) of the human sequence that have no amino acids corresponding to amino acids present in mouse GM-CSF, shown below, and indicated with a vertical line. There are also several amino acids, indicated by asterisks, present only in the human sequence.

and macrophage colonies. While the predominant colony types appear to be neutrophilic granulocytes and macrophages or mixtures of these two cell types (Fig. 5 *Lower*), in some experiments a small fraction (3–14%) of the colonies contain eosinophils. Comparable results were obtained when the supernatants were assayed with human bone marrow or cord blood cells. However, when COS-7 supernatants for representative clones were assayed on mouse bone marrow cultures, no activity was detected (Table 2).

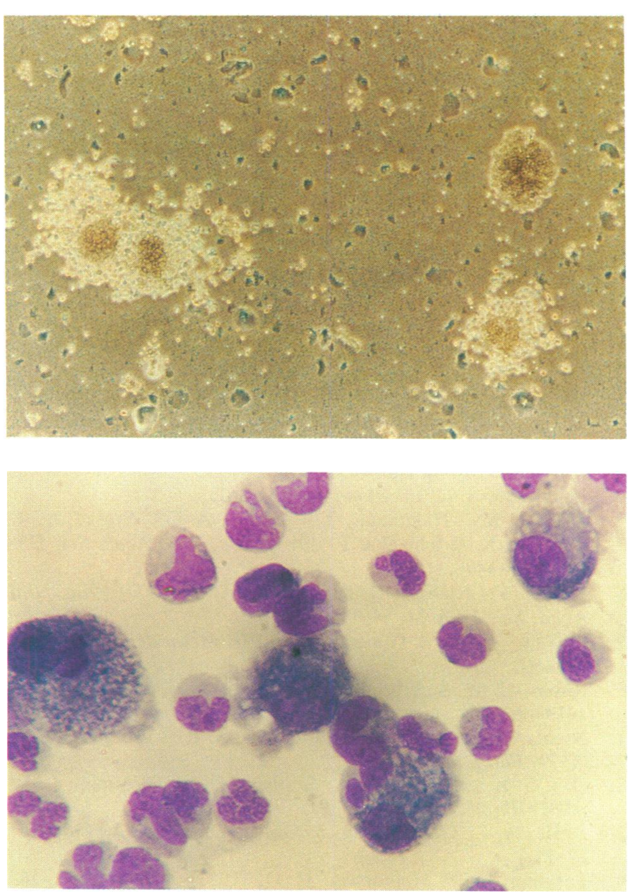

FIG. 5. (*Upper*) Macroscopic appearance of bone marrow colonies stimulated by human GM-CSF. (×40.) (*Lower*) Cells from colonies were stained with Wright–Giemsa, showing neutrophils and macrophages. (×40.)

DISCUSSION

Several human CSFs from different sources have been described with activity for granulocytes and macrophages (21, 22). The GM-CSF activities in human placental conditioned medium have been further resolved into two fractions. One, designated CSF-α, stimulates macrophage and neutrophilic and eosinophilic granulocyte colonies. The other, termed CSF-β, stimulates exclusively neutrophil and macrophage colonies (21). These prior studies could not distinguish whether CSF-α or CSF-β were single biochemical entities or whether each was composed of more than one factor; in particular, it was unclear whether CSF-α could be composed of a neutrophil/macrophage CSF copurifying with an eosinophil CSF (21). A similar factor, partially purified from a T-lymphoblast cell line (Mo), has also been described

Table 3. Cellular composition of colonies stimulated by supernatants of transfected cells

	Cell composition, %					
Exp.	Neu	Mϕ	Eos	Neu/Mϕ	Mϕ/Eos	Neu/Mϕ/Eos
1	14	24	3	53	1	5
2	11	31	0	55	0	3
3	18	29	4	39	1	9

Cellular compositions of cord blood colonies (Exps. 1 and 2) or bone marrow colonies (Exp. 3) stimulated by cell supernatants from COS-7 cells transfected with human GM-CSF cDNA clone are tabulated. A minimum of 70 colonies was analyzed from each experiment. Cells in colonies composed of more than 150 cells were identified after staining with Wright–Giemsa. Results are presented as the percentage of each pure or mixed colony type. Neu, neutrophil; Mϕ, macrophage; Eos, eosinophil.

as having activity on eosinophil colonies in addition to neutrophil and macrophage colonies (23).

In this report we describe the isolation of cDNA clones that encode a human colony-stimulating factor expressed in Con A-activated human T cells. Functional clones were identified by transfecting COS-7 cells with randomly picked cDNA clones and measuring the CSF activity secreted into the cell supernatant. Independently, other functional cDNA clones were identified on the basis of cross-hybridization with a probe prepared from a murine GM-CSF cDNA. We determined that all the active clones identified by either procedure are essentially identical, and the homology between the human and mouse cDNA clones first detected by hybridization was confirmed by nucleotide sequence analysis. These results indicate that transfection of random or selected cDNA clones into mammalian cells coupled with an *in vitro* bone marrow or cord blood assay may lead to the identification of additional clones encoding human hematopoietic growth factors.

The nucleotide sequence of the human GM-CSF cDNA clone exhibits a strong homology in both the coding and non-coding regions with that of mouse GM-CSF. Comparison of the nucleotide sequences reveals approximately 70% homology, while the deduced amino acid sequences share approximately 50% homology (Fig. 4; ref. 7; unpublished results). The single long open reading frame in the human cDNA clone consists of 144 amino acid residues. The native protein from human T cells or other sources has not as yet been purified to homogeneity and no amino acid sequence information is available. Because this CSF is a secreted protein, a hydrophobic leader sequence would be expected to precede the sequence for the mature secreted form of the protein. There is significant amino acid sequence homology between the murine and human GM-CSF sequences at the amino terminus (Fig. 4). Analysis of the hydrophobicity of the polypeptide and comparison with a proposed consensus sequence for the processing of signal peptides (24) suggest that cleavage of the precursor polypeptide would occur following the alanine residue at position 20 (Fig. 4). We would therefore predict that the mature polypeptide would be 124 amino acid residues long and begin with an arginine residue. The amino acid homology with the murine sequence appears to be distributed throughout the molecule, in addition to the hydrophobic leader sequence.

The deduced molecular weight of secreted GM-CSF is approximately 14,000. This predicted molecular weight does not take into account potential post-translational glycosylation of the polypeptide, which is predicted by the presence of two potential N-glycosylation sequences (see Fig. 3). Despite the high degree of homology between human GM-CSF and murine GM-CSF, the expressed human gene product has no activity on murine bone marrow cells. This result is also consistent with the properties of the GM-CSF characterized from a T lymphoblast cell line (23).

The CSF encoded by the cDNA clones described here stimulates the development of neutrophil and macrophage colonies when incubated with human bone marrow or cord blood cells. The activity of the gene product expressed in COS-7 cells also suggests that this human CSF has a minor stimulatory effect on eosinophil precursors. All of these properties would correspond well with one class of GM-CSF described previously, particularly with respect to the activity on eosinophil progenitor cells (21–23). However, additional studies using the cloned and expressed gene product will be necessary to establish whether all these activities are due to direct effects on individual progenitor populations. Further, there may exist one or more classes of human GM-CSFs with activity specific only for neutrophils and macrophages, as has been described for CSF-β (21). The cloning and expression of this and other human CSFs will help to clarify the precise number of soluble factors involved in human hematopoiesis and provide a means for obtaining purified material for further biological and biochemical characterization.

Note Added in Proof. Gasson *et al.* (25) recently published a partial NH_2-terminal sequence of GM-CSF purified from Mo T cells; comparison with Fig. 3 suggests that cleavage of the precursor polypeptide occurs after the serine residue at position 17.

We thank Gary Nabel for providing Con A-activated T cell clone T7; Jacques Banchereau and Helmut Pohlit for clone 5C10 and peripheral blood lymphocytes; and Nelson Teng and Lee Levitt for cord blood and human bone marrow, respectively. We acknowledge the assistance of Ellen Jacob, Barbara Daine, and Thyra Ilmer with DNA transfections, Elizabeth Baheri and Michelle Schwartz with the screening of the libraries, and Sandra Zurawski for DNA sequencing. Albert Zlotnik, James Ihle, and David Nathan provided useful discussions.

1. Schrader, J. W., Clark-Lewis, I. & Barllett, P. F. (1982) in *Lymphokines*, ed. Pick, E. 5, (Academic, New York), Vol. 5, pp. 291–321.
2. Nabel, G., Greenberger, J. S., Sakakeeny, M. A. & Cantor, H. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1157–1161.
3. Prystowsky, M. B., Ely, J. M., Beller, D. I., Eisenberg, L., Goldman, J., Goldman, M., Goldwasser, E., Ihle, J. N., Quintana, J., Remold, H., Vogel, S. N. & Fitch, F. W. (1982) *J. Immunol.* **129**, 2337–2344.
4. Metcalf, D. (1981) in *Tissue Growth Factors*, ed. Baserga, R. (Springer, New York), pp. 343–384.
5. Burgess, A. (1984) *Growth Factors and Stem Cells* (Academic, New York), pp. 93–124.
6. Burgess, A. W. & Metcalf, D. (1980) *Blood* **56**, 947–958.
7. Gough, N. M., Gough, J., Metcalf, D., Kelso, A., Grail, D., Nicola, N. A., Burgess, A. W. & Dunn, A. R. (1984) *Nature (London)* **309**, 763–767.
8. Yokota, T., Lee, F., Rennick, D., Hall, C., Arai, N., Mosmann, T., Nabel, G., Cantor, H. & Arai, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1070–1074.
9. Fung, M. C., Hapel, A. J., Ymer, S., Cohen, D. R., Johnson, R. M., Campbell, H. D. & Young, I. G. (1984) *Nature (London)* **307**, 233–237.
10. Rennick, D., Lee, F., Yokota, T., Arai, K., Cantor, H. & Nabel, G. J. (1985) *J. Immunol.* **134**, 910–914.
11. Lusis, A. J., Golde, D. W., Quan, D. H. & Lasky, L. A. (1982) *Nature (London)* **298**, 75–77.
12. Yokota, T., Arai, N., Lee, F., Rennick, D., Mosmann, T. & Arai, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 68–72.
13. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
14. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 280–289.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Knudtzou, S. (1974) *Blood* **43**, 357–361.
17. Ogawa, M., Nakahata, T., Leary, A. G., Sterk, A. R., Ishizaka, K. & Ishizaka, T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4494–4498.
18. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
19. Maxam, A. W. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
20. Rubin, C. M. & Schnid, C. W. (1980) *Nucleic Acids Res.* **8**, 4613–4619.
21. Nicola, N. A., Metcalf, D., Johnson, G. R. & Burgess, A. W. (1979) *Blood* **54**, 614–627.
22. Das, S. K., Stanley, E. R., Guilbert, L. J. & Forman, L. W. (1981) *Blood* **58**, 630–641.
23. Lusis, A. J., Quan, D. H. & Golde, D. W. (1981) *Blood* **57**, 13–21.
24. Perlman, D. & Halvorson, H. O. (1983) *J. Mol. Biol.* **167**, 391–409.
25. Gasson, J. C., Weisbart, R. H., Kaufman, S. E., Clark, S. C., Hewick, R. M., Wong, G. G. & Golde, D. W. (1984) *Science* **226**, 1339–1342.